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TITLE: Method of nucleic acid sequence selection

BSPR:

A DNA isolation method, termed "triplex affinity capture," has been described in which a specific double-stranded genomic DNA is hybridized to a biotinylated homopyrimidine oligonucleotide probe to form a "triplex complex," which can then be selectively bound to streptavidin-coated magnetic beads (Ito, T. et al., Nucleic Acids Res. 20: 3524 (1992); Ito, T. et al., Proc. Natl. Acad. Sci. (U.S.A.) 89: 495-498 (1992)). Takabatake, T. et al. have described a variation of this technique that employs a biotinylated purine-rich oligonucleotide probe to detect and recover the desired nucleic acid molecule (Takabatake, T. et al., Nucleic Acid Res. 20: 5853-5854 (1992)). A practical drawback with these particular approaches is that they are restricted to isolation of target DNA sequences containing homopurine-homopyrimidine tracts.

DEPR:

As a consequence of such binding, any biotinylated probe that has hybridized to a desired target molecule will become bound to the bead or support. In contrast, non-target molecules will remain unbound, and can be separated from the bound material by washing, filtration, centrifugation, sieving, or by magnetic separation methods. Most preferably, however, a magnet is used to pull the paramagnetic bead out of solution, and the beads are washed with a suitable buffer (such as one containing Tris, EDTA, and NaCl). Such treatment removes the majority of non-target nucleic acid sequences that were originally present, and hence eliminates undesired non-selected single-stranded phagemid DNA from the reaction.

DEPR:

The specifically captured single-stranded phagemid target DNA (hybridized to the biotinylated probe) is then released from the probe by treatment such as addition of an alkaline buffer, heat, etc. In a preferred sub-embodiment, the selected target DNA is resuspended in a formamide-Tris-EDTA buffer and released from the beads by heating at a temperature of 60.degree.-70.degree. C. for a short period of time. The releasing treatment is preferably selected such that the biotinylated probe remains attached to the magnetic beads, which is then removed from solution by sieving, centrifugation, filtration, or more preferably, by a magnet.

DEPR:

The oligonucleotide probes were biotin-labeled using biotin-14-dCTP and terminal deoxynucleotidyl transferase (TdT) as described by Flickinger, J. L. et al. (Nucleic Acids Res. 20: 2382 (1992)) with the following minor modifications. In a typical reaction, 0.3-0.5 nmol (.apprxeq.5 .mu.g) of oligonucleotides (21-25-mer), 500 .mu.M of biotin-14-dCTP and 60 units of TdT in 50 .mu.l of 1.times. tailing buffer [100 mM potassium cacodylate (pH 7.2), 2 mM CoCl.sub.2 and 200 .mu.M DTT] was incubated at 37.degree. C. for 15 min. The reaction was terminated by adding 2 .mu.l of 0.25M EDTA. The labeled probes were precipitated by adding an equal volume (52 .mu.l) of 1M Tris buffer (pH 7.5), 10 .mu.g glycogen as carrier, and 2.5 volume (260 .mu.l) of ethanol, and stored on dry ice for 10 min. After centrifugation at 4.degree. C. for 10 min, the probes were rinsed with 100 .mu.l of 75% ethanol and centrifuged for 2 min. The probes were air-dried and dissolved in 10 .mu.l of TE. To determine the labeling efficiency and the concentration of the labeled probe, 2 .mu.l of labeled products were resuspended in an equal volume of sequencing reaction stop buffer [95% (v/v) formamide, 10 mM EDTA (pH 8.0), 0.1% (w/v) bromophenol blue, 0.1% (w/v) xylene

cyanol], heated at 95.degree. C. for 1 min and chilled on ice. The probes were electrophoresed along with a known amount of the starting material on 16% denaturing PAGE. The gel was soaked in a ethidium bromide solution (0.5 .mu.g/ml) for 15 min, and photographed. Typically, more than 95% of the oligonucleotide will be labeled. The concentration of the labeled probes was determined by the comparison to the known starting material.

DEPR:

The hybridization was performed by the following procedure: 1-10 .mu.g of single-stranded target library DNA was diluted with 10 .mu.l of dilution buffer (100 mM HEPES, pH 7.5, 2 mM EDTA and 0.2% SDS) to a final volume of 19 .mu.l in a 5 ml Falcon tube. The DNA was denatured at 95.degree. C. for 1 min and immediately chilled in ice water for 5 min. 1 .mu.l (20 ng) of biotin-probe was added to the DNA mixture, followed by the addition of 5 .mu.l of 5M NaCl. The hybridization mixture was incubated at 42.degree. C. with continuous shaking (200 rpm) in a culture incubator for 24 h. Before binding the hybrids to the streptavidin, 50 .mu.l of the streptavidin coated paramagnetic beads (DYNAL) were washed once with 1.times. binding buffer (10 mM TRIS, pH 7.5, 1 mM EDTA and 1M NaCl) by following the manufacturer's instructions. The paramagnetic beads were resuspended in 20 .mu.l of 1.times. binding buffer. The hybridization mixture was added to the resuspended beads and mixed well. The mixture was incubated at room temperature for 1 h with occasional mixing by gently tipping the tube. The paramagnetic beads were separated from the DNA bulk by inserting the tube into the magnet, and washed 6 times with the washing buffer (10 mM Tris, pH 7.5, 1 mM EDTA and 500 mM NaCl). Finally, the paramagnetic beads were resuspended in 20 .mu.l of 30% formamide in TE buffer. The selected DNA was released by heating the beads at 65.degree. C. for 5 min. The tube was inserted into the magnet, and the aqueous phase was transferred to a new tube. The beads were washed once with 15 .mu.l of TE buffer, and the aqueous phases were pooled. The selected DNA was precipitated with 0.5 volumes of 7.5M ammonium acetate, 10 .mu.g of glycogen, and 2.5 volume of ethanol. The DNA pellet was dissolved in 5-10 .mu.l of TE buffer. An aliquot (1 .mu.l) was used for the electroporation to determine the hybrid selection efficiency.

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